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PEPTIDES USEFUL AS INHIBITORS OF RENIN FOR TREATING HYPERTENSION AND HYPERALDOSTERONISM

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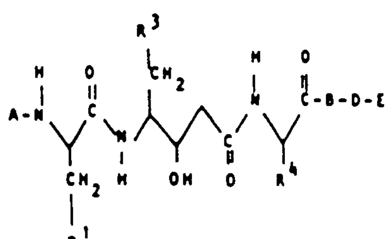
(54) **Renin inhibitory tripeptides.**

(57) Disclosed are Renin inhibitory peptides of the formula



(I.)

wherein
A is, e.g., hydrogen;
R^1 is, e.g., hydrogen;
R^3 is e.g., C_{3-6} alkyl;
R^4 is e.g., hydrogen; or

$$-\underset{\displaystyle R^7}{\underset{|}{CH}}-R^6,$$

where R^7 is hydrogen; C_{1-4} alkyl; aryl; aryl substituted with up to three members selected from the group consisting of C_{1-4} alkyl, trifluoromethyl, hydroxy, C_{1-4} alkoxy, fluoro, chloro, bromo, and iodo; or indolyl;
R^6 is hydrogen; C_{1-4} alkyl; hydroxy; or C_{3-7} cycloalkyl; and
B is, e.g., $-Y-(CH_2)_n-R^8$ where Y is $-NH-$ or $-O-$; n is 0 to 5; and
R^8 is, e.g., hydrogen;
D is, e.g., absent; glycyl; or sarcosyl; and
E is absent; OR; NHR; or $N(R)_2$, where R may be the same or different and is hydrogen or C_{1-4} alkyl;
wherein all of the asymmetric carbon atoms have an *S* configuration, except for those in the A, B and D substituents, which may have an *S* or *R* configuration; and a pharmaceutically acceptable salt thereof.

Those compounds inhibit renin and are useful for treating various forms of renin-associated hypertension and hyperaldosteronism.

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TITLE OF THE INVENTION

RENIN INHIBITORY TRIPEPTIDES

BACKGROUND OF THE INVENTION

1. Field of The Invention

The present invention is concerned with novel peptides which inhibit renin.

The present invention is also concerned with pharmaceutical compositions containing the novel peptides of the present invention as active ingredients, with methods of treating renin-associated hypertension and hyperaldosteronism, with diagnostic methods which utilize the novel peptides of the present invention, and with methods of preparing the novel peptides of the present invention.

Renin is a proteolytic enzyme of molecular weight about 40,000, produced and secreted by the kidney. It is secreted by the juxtaglomerular cells and acts on the plasma substrate, angiotensinogen, to split off the decapeptide angiotensin I, which is

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converted to the potent pressor agent angiotensin II. Thus, the renin-angiotensin system plays an important role in normal cardiovascular homeostasis and in some forms of hypertension.

In the past, attempts to modulate or manipulate the renin-angiotensin system have met with success in the use of inhibitors of angiotensin I converting enzyme. In view of this success, it seems reasonable to conclude that a specific inhibitor of the limiting enzymatic step that ultimately regulates angiotensin II production, the action of renin on its substrate, would be at least equally successful. Thus, an effective inhibitor of renin has been long sought as both a therapeutic agent and as an investigative tool.

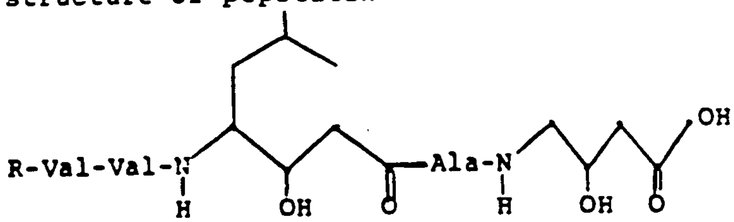
2. Brief Description of the Prior Art

There has been substantial interest in the synthesis of useful renin inhibitors for many decades; and the following table lists the major classes of renin inhibitors that have been studied, as well as their inhibition constants (K_i):

Class	K_i (M)
Renin antibody	probably 10^{-6}
Pepstatin	10^{-6} - 10^{-7}
Phospholipids	10^{-3}
Substrate analogs	
Tetrapeptides	10^{-3}
Octa- to tridecapeptides	10^{-5} - 10^{-6}

Umezawa et al., in J. Antibiot. (Tokyo) 23: 259-262, 1970, reported the isolation of a peptide from actinomyces that was an inhibitor of aspartyl proteases such as pepsin, cathepsin D, and renin.

This peptide, known as pepstatin, was found by Gross et al., Science 175:656, 1971, to reduce blood pressure in vivo after the injection of hog renin into nephrectomized rats. However, pepstatin has not found wide application as an experimental agent because of its limited solubility and its inhibition of a variety of other acid proteases in addition to renin. The structure of pepstatin is shown below:



To date, many efforts have been made to prepare a specific renin inhibitor based on substrate analogy. Since the human renin substrate has only recently been elucidated (Tewksbury et al., Circulation 59, 60, Supp. II: 132, Oct. 1979), heretofore substrate analogy has been based on the known pig renin substrate. While the human and pig renin substrates are not the same, the substrate analogy based on pig renin has always been considered acceptable in the art as predictive of human renin inhibitory activity because of the closely related activity of the two renins. Thus, while pig renin does not cleave the human renin substrate, human renin, on the other hand, does cleave the pig renin substrate. See Poulsen et al., Biochim. Biophys. Acta 452:533-537, 1976; and Skeggs, Jr. et al., J. Exp. Med. 106:439-453, 1957. Moreover, the human renin inhibit ry activity f the peptid s of the present

invention most active in inhibiting pig renin has been confirmed, thus providing further evidence of this accepted correlation between human and pig renin activity.

It has been found, for example, using pig renin substrate analogy, that the octapeptide sequence extending from histidine-6 through tyrosine-13 has kinetic parameters essentially the same as those of the full tetradecapeptide renin substrate. The amino acid sequence of the octapeptide in pig renin substrate is as follows:

6	7	8	9	10	11	12	13
-His-	Pro-	Phe-	His-	Leu-	Leu-	Val-	Tyr-

Renin cleaves this substrate between Leu^{10} and Leu^{11}.

Kokubu et al., Biochem. Pharmacol. 22: 3217-3223, 1973, synthesized a number of analogs of the tetrapeptide found between residues 10 to 13, but while inhibition could be shown, inhibitory constants were only of the order of 10^{-3}M.

Analogs of a larger segment of renin substrate were also synthesized: Burton et al., Biochemistry 14: 3892-3898, 1975, and Poulsen et al., Biochemistry 12: 3877-3882, 1973. Two of the major obstacles which had to be overcome to obtain an effective renin inhibitor useful in vivo were lack of solubility and weak binding (large inhibitory constant). Modifications to increase solubility soon established that the inhibitory properties of th peptides are markedly dependent on the hydrophobicity of various amino acid residues, and that increasing

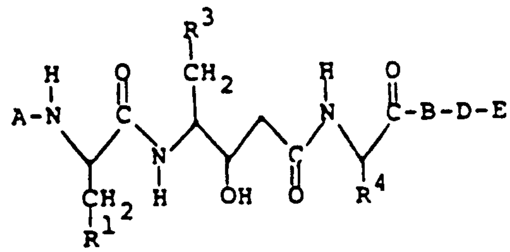
solubility by replacing lipophilic amino acids with hydrophilic isosteric residues becomes counter-productive. Other approaches to increasing solubility have had limited success. Various modifications designed to increase binding to renin have also been made, but here too, with only limited success. For a more detailed description of past efforts to prepare an effective inhibitor of renin, see Haber and Burton, Fed. Proc. Fed. Am. Soc. Exp. Biol. 38: 2768-2773, 1979.

More recently, Hallett, Szelke, and Jones, in work described in European Patent Publication No. 45,665 Nature, 299, 555 (1982), and Hypertension, 4, Supp. 2, 59 (1981), have replaced the Leu-Leu site of renin cleavage by isosteric substitution, and obtained compounds with excellent potentcy.

For other articles describing previous efforts to devise renin inhibitors, see Marshall, Federation Proc. 35: 2494-2501, 1976; Burton et al., Proc. Natl. Acad. Sci. USA 77: 5476-5479, Sept. 1980; Suketa et al., Biohemistry 14: 3188, 1975; Swales, Pharmac. Ther. 7: 173-201, 1979; Kokubu et al., Nature 217: 456-457, Feb. 3, 1968; Matsushita et al., J. Antibiotics 28: 1016-1018, Dec. 1975; Lazar et al., Biochem. Pharma. 23: 2776-2778, 1974; Miller et al., Biohem. Pharma. 21: 2941-2944, 1972; Haber, Clinical Science 59:7s-19s, 1980; and Rich et al., J. Org. Chem. 43: 3624, 1978, and J. Med. Chem. 23: 27, 1980.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

In accordance with the present invention there are provided renin inhibitory peptides of the formula:



(I.)

wherein:

A is hydrogen; or $R_a^2-\underset{R_b^2}{\underset{|}{X}}-\overset{O}{\overset{\|}{C}}-$

where

X is -O-; -O-CH-; -CH-O-; $-\underset{|}{CH}-$; $-NH-\underset{|}{CH}-$; or $-S-\underset{|}{CH}-$; and

R_a^2 and R_b^2 may be the same or different and are hydrogen; $Y-(CH_2)_n-$ or $Y-(CH_2)_m-CH{=}CH-(CH_2)_p-$, where Y is hydrogen; aryl; aryl substituted with up to five members independently selected from the group consisting of C_{1-8}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, and halo;

n is 0 to 5; m is 0 to 2; and p is 0 to 2; except that where X is -O-, only one of R_a^2 or R_b^2 is present;

R^1 is hydrogen; C_{1-4} alkyl, provided, that where R^1 is i-propyl, and B is Phe or Tyr, A is other than hydrogen or phenoxyacetyl; hydroxy C_{1-4} alkyl;

aryl; aryl substituted with up to three

members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy; halo; indolyl; 4-imidazolyl; amino C_{2-4} alkyl; guanidyl C_{2-3} alkyl; or methylthiomethyl;

R^3 is C_{3-6} alkyl; C_{3-7} cycloalkyl; aryl; or C_{3-7}cycloalkyl or aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, and halo;

R^4 is hydrogen; or $-\underset{\displaystyle R^2}{\underset{|}{CH}}-R^5$, where R^2 is hydrogen; C_{1-4}alkyl; aryl; aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, and halo; or indolyl; and R^5 is hydrogen; C_{1-4} alkyl; hydroxy; or C_{3-7} cycloalkyl; and

B is (1) $-Y-(CH_2)_n-R^6$

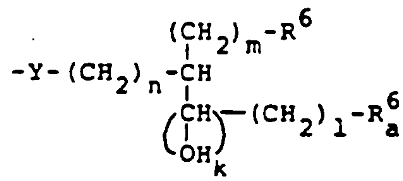
where

Y is -NH- or -O-;

n is 0 to 5; and

R^6 is hydrogen; hydroxy; C_{1-4}alkyl; C_{3-7}cycloalkyl; aryl; aryl substituted with up to five members independently selected from the group consisting of C_{1-6}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, amino, mono- or di- C_{1-4} alkylamino, and halo; amino; mono-,

di-, or tri-C_{1-4}alkylamino; guanidyl; heterocyclic; or heterocyclic substituted with up to five members independently selected from the group consisting of C_{1-6}alkyl, hydroxy, trifluoromethyl, C_{1-4}alkoxy, halo, aryl, aryl C_{1-4}alkyl, amino, and mono- or di-C_{1-4}alkylamino;

(2) 

where

Y is as defined above;

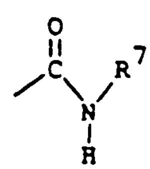
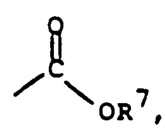
n is 0 or 1;

k is 0 or 1;

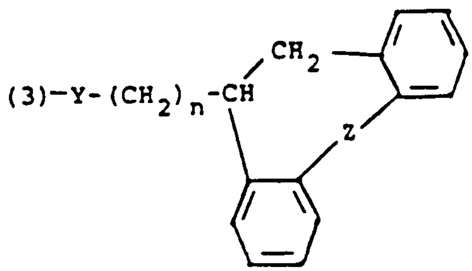
l is 1 to 4;

m is 1 to 4; and

R^6 and R^6_a may be the same or different and have the same meaning as R^6 above and R^6_a may additionally be

 or 

where R^7 is hydrogen or C_{1-3}alkyl;



where

Y is as defined above;

n is 0 or 1; and

Z is

(a) $-(CH_2)_n-\underset{R^7}{\underset{|}{CH}}-$

where

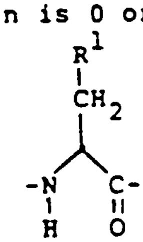
n is 0 or 1; and

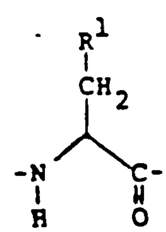
R^7 is as defined above; or

(b) $-(CH_2)_n-\underset{CH_2}{\underset{\|}{C}}-$

where

n is 0 or 1; or

(4)  ;

D is absent; glycyl; sarcosyl; or  ; and

E is absent; OR; NHR; or $N(R)_2$, where R may be th same or different and is hydrogen or C_{1-4}alkyl;

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wherein all of the asymmetric carbon atoms have an S configuration, except for those in the A ~~and~~ B, and D substituents, which may have an S or R configuration; and a pharmaceutically acceptable salt thereof.

While both the S and R chiralities for asymmetric carbon atoms in the B substituent are included in the peptides of the present invention, preferred chiralities are indicated in the description which follows.

In the above definitions, the term "alkyl" is intended to include both branched and straight chain hydrocarbon groups having the indicated number of carbon atoms.

The term "halo" means fluoro, chloro, bromo and iodo.

The aryl substituent represents phenyl, and naphthyl.

The heterocyclic substituent recited above represents any 5- or 6-membered aromatic ring containing from one to three heteroatoms selected from the group consisting of nitrogen, oxygen, and sulfur; having various degrees of saturation; and including any bicyclic group in which any of the above heterocyclic rings is fused to a benzene ring. Heterocyclic substituents in which nitrogen is the heteroatom are preferred, and of these, those containing a single nitrogen atom are preferred. Fully saturated heterocyclic substituents are also preferred. Thus, piperidine is a preferred heterocyclic substituent. Other preferred heterocyclic substituents are: pyrryl, pyrrolinyl, pyrrolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyridyl,

piperidinyl, pyrazinyl, piperazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isoxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, isothiazolidinyl, indolyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, furyl, thienyl and benzothienyl.

Where the heterocyclic substituent itself is substituted, it is preferred that the substituent be arylC_{1-4}alkyl.

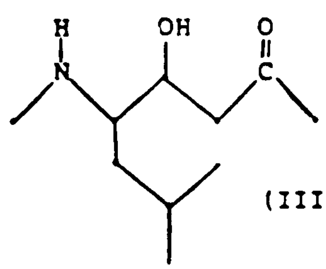
The novel renin inhibitory peptides of the present invention may also be described in terms of common amino acid components and closely related analogs thereof, in accordance with the following formula:

A-F-Sta-G-B-D-E

(II.)

The A,B,D, and E components correspond to the same portions of Formula I.

In Formula II, Sta represents the unusual amino acid statine and its closely related analogs, and its presence constitutes a unique feature of the renin inhibitory peptides of the present invention. Statine may be named as 4(S)-amino-3(S)-hydroxy-6-methylheptanoic acid, and may be represented by the following formula:



(III)

As shown in Formula III above, the delta-substituent in naturally-occurring statine is isopropyl, or a leucine sidechain, essentially. As shown in Formula I by the R^3 substituents, the isopropyl group may be replaced by higher alkyl groups up to six carbon atoms, cycloalkyl groups containing from three to seven carbon atoms, aryl, and C_{3-7}cycloalkyl or aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, fluoro, chloro, bromo, and iodo. A phenyl substituent is especially preferred. These modifications of the naturally-occurring statine structure are in accordance with the hydrophobicity considered necessary to maintain the inhibitory activity of the total peptide.

The remaining common amino acid components of Formula II are as follows:

A has the same meaning as above in Formula I;

F is Ala, Leu, Phe, Tyr, Trp, His, Lys, Orn, Arg, or Met;

G is Ala, Leu, Phe, Tyr, Trp, Ser, Gly, Val, Ile, or Thr; and

B, D, and E have the same meaning as above in Formula I.

It will be understood that closely related analogs of the above common amino acids, for example, aliphatic amino acids in addition to Ala, Val, Leu, and Ile, such as α-aminobutyric acid (Abu), and

substituted phenyl derivatives of Phe, are included in the broad description of the novel inhibitory peptides of the present invention represented by Formula I and its definitions. Thus, the peptides of Formula II and its definitions, including the derivatives of naturally-occurring statine represented by the definitions of the R^3 substituent in Formula I, represent preferred peptides of the present invention.

Preferred inhibitory peptides of the present invention are the following:

His-Sta-Leu-Phe-methyl ester;
His-Sta-Phe-Phe-methyl ester;
His-Sta-Ala-Phe-methyl ester;
BOC[1]-His-Sta-Leu-Phe-amide;
POA[2]-His-Sta-Leu-Phe-methyl ester;
POA-His-Sta-Leu-Phe-amide;
POA-His-Sta-Phe-Phe-amide;
3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-methyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-ethyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2,3-diphenylpropionyl-His-Sta-Leu-benzylamide;
2-benzyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenethyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenylpropyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenylbutyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenethenyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-(4,4-dimethyl-1-butenyl)-3-phenylpropionyl-His-Sta-Leu-benzylamide;
10,11-dihydro-5H-dibenzo-[2,d]cycloneptenylcarbonyl-His-Sta-Leu-benzylamide;
BOC -His-AHPPA[3]-Leu-benzylamide;
3-phenylpropionyl-His-AHPPA-Leu-benzylamide;
POA-His-AHPPA-Leu-benzylamide;
2-benzyl-3-phenylpropionyl-His-AHPPA-Leu-benzylamide.

POA-His-ACHPA[4]-Leu-Phe-amide;
POA-His-ACHPA-Ile-benzylamide;
POA-His-ACHPA-Ile-2-pyridylmethylamide;
2-phenylpropyl-3-phenylpropionyl-His-AHPPA-Leu-benzyl-amide.

Especially preferred inhibitory peptides of the present invention are the following:

POA-His-Sta-Leu-Phe-amide;
POA-His-AHPPA-Leu-benzylamide;
2-phenylpropyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenylpropyl-3-phenylpropionyl-His-AHPPA-Leu-benzylamide;
2-benzyl-3-phenylpropionyl-His-AHPPA-Leu-benzylamide.
POA-His-ACHPA[4]-Leu-Phe-amide;
POA-His-ACHPA-Ile-benzylamide;
POA-His-ACHPA-Ile-2-pyridylmethylamide;
2-phenylpropyl-3-phenylpropionyl-His-AHPPA-Leu-benzyl amide.

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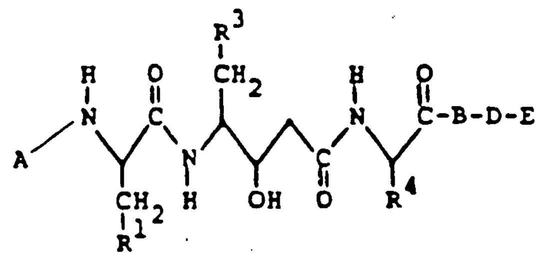
[1] BOC = _Tert_-butyloxycarbonyl.
[2] POA = Phenoxyacetyl.
[3] AHPPA = (3S, 4S)-4-Amino-3-hydroxy-5-phenyl-pentanoic acid.
[4] ACHPA = (3S, 4S)-4-Amino-5-cyclohexyl-3-hydroxy-pentanoic acid.

The inhibitory peptides of the present invention may be better appreciated in terms of substrate analogy from the following illustration of Formula I alongside the octapeptide sequence of a portion of the pig renin substrate, which renin cleaves between Leu^{10} and Leu^{11}:

Pro	Phe	His	Leu	Leu	Val	Tyr	
7	8	9	10	(11)	12	13	(14)

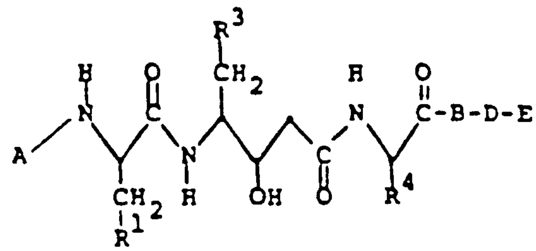


Statine

As can be seen, a unique aspect and essential feature of the present invention is the substitution of the single statine amino acid component for the double amino acid sequence: Leu^{10}-Leu^{11} in the endogenous pig renin substrate. It is believed that substitution of statine for both leucine amino acids rather than just one leucine results in an improved substrate analogy due to the greater linear extent of statine as compared to a single leucine component. Thus, statine more closely approximates Leu-Leu in linear extent, and thereby provides a better "fit" to the renin enzyme.

The inhibitory peptides of the present invention may also be better appreciated in terms of substrate analogy from the following illustration of Formula I alongside the octapeptide sequence of a portion of the human renin substrate, which renin cleaves between Leu^{10} and Val^{11}:

Pro	Phe	His	Leu	Val	Ile	His	
7	8	9	10	(11)	12	13	(14)



Statine

As can be seen, a unique aspect and essential feature of the present invention is the substitution of the single statine amino acid component for the double amino acid sequence: Leu^{10}-Val^{11} in the endogenous human renin substrate. It is believed that substitution of statine for both the leucine and valine amino acids rather than just the leucine results in an improved substrate analogy due to the greater linear extent f statine as compared to a single leucine component. Thus, statine more closely approximates Leu-Val in linear extent, and thereby provides a better "fit" to the human renin enzyme.

In the endogenous substrate it is also preferred to substitute Leu for Val^{12} and Phe for Tyr^{13} in order to enhance the inhibitory activity of the resulting peptide.

The Formula I compounds can be used in the form of salts derived from inorganic or organic acids and bases. Included among such acid addition salts are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dod cylsulfate,

ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate, and undecanoate. Base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases such as dicyclohexylamine salts, N-methyl-D-glucamine, and salts with amino acids such as arginine, lysine, and so forth. Also, the basic nitrogen-containing groups can be quaternized with such agents as lower alkyl halides, such as methyl, ethyl, propyl, and butyl chloride, bromides and iodides; dialkyl sulfates like dimethyl, diethyl, dibutyl; and diamyl sulfates, long chain halides such as decyl, lauryl, myristyl and stearyl chlorides, bromides and iodides, aralkyl halides like benzyl and phenethyl bromides and others. Water or oil-soluble or dispersible products are thereby obtained.

The novel peptides of the present invention possess an excellent degree of activity in treating renin-associated hypertension and hyperaldosteronism.

For these purposes the compounds of the present invention may be administered parenterally, by inhalation spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used herein includes subcutaneous injections, intravenous, intramuscular,

intrasternal injection or infusion techniques. In addition to the treatment of warm-blooded animals such as mice, rats, horses, dogs, cats, etc., the compounds of the invention are effective in the treatment of humans.

The pharmaceutical compositions may be in the form of a sterile injectable preparation, for example as a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectibles.

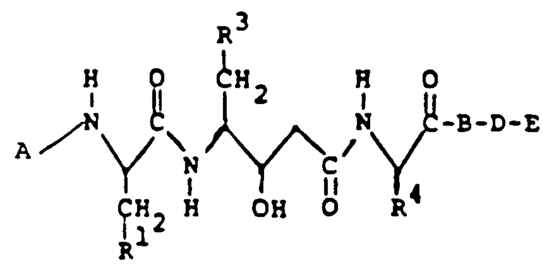
The peptides of this invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

Dosage levels of the order of 2 to 35 grams per day are useful in the treatment of the above indicated conditions. For example, renin-associated hypertension and hyperaldosteronism are effectively treated by the administration of from 30 milligrams to 0.5 grams of the compound per kilogram of body weight per day.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

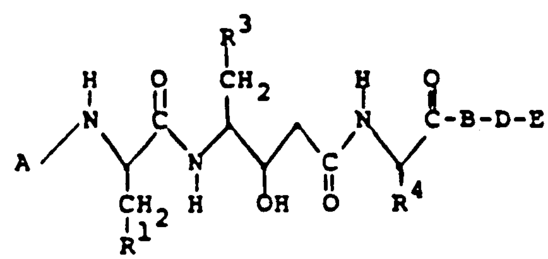
It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

Thus, in accordance with the present invention there is further provided a pharmaceutical composition for treating renin-associated hypertension and hyperaldosteronism, comprising a pharmaceutical carrier and a therapeutically effective amount of a peptide of the formula:



wherein A, R^1, R^3, R^4, B, D, and E have the same meaning as recited further above for Formula I; wherein all of the asymmetric carbon atoms have an S configuration, except for those in the A and B substituents, which may have an S or R configuration; and a pharmaceutically acceptable salt thereof.

Also, in accordance with the present invention there is still further provided a method of treating renin-associated hypertension and hyperaldosteronism, comprising administering to a patient in need of such treatment, a therapeutically effective amount of a peptide of the formula:



wherein A, R^1, R^3, R^4, B, D, and E have the same meaning as recited further above for Formula I; wherein all of the asymmetric carbon atoms have an S configuration, except for those in the A and B substituents, which may have an S or R configuration; and a pharmaceutically acceptable salt thereof.

The renin inhibitory novel peptides of the present invention may also be utilized in diagnostic methods for the purpose of establishing the significance of renin as a causative or contributory factor in hypertension or hyperaldosteronism in a particular patient. For this purpose the novel peptides of the present invention may be administered

in a single dose of from 0.1 to 10 mg per kg of body weight.

Both in vivo and in vitro methods may be employed. In the in vivo method, a novel peptide of the present invention is administered to a patient, preferably by intravenous injection, although parenteral administration is also suitable, at a hypotensive dosage level and as a single dose, and there may result a transitory fall in blood pressure. This fall in blood pressure, if it occurs, indicates supranormal plasma renin levels.

An in vitro method which may be employed involves incubating a body fluid, preferably plasma, with a novel peptide of the present invention and, after deproteinization, measuring the amount of angiotensin II produced in nephrectomized, pentolinium-treated rats. Another in vitro method involves mixing the plasma or other body fluid with a novel peptide of the present invention and injecting the mixture into a test animal. The difference in pressor response with and without added peptide is a measure of the renin content of the plasma.

Pepstatin may be employed in the methods described above as an active control. See, e.g., U.S. Patent Nos. 3,784,686 and 3,873,681 for a description of the use of pepstatin in diagnostic methods of this type.

The novel peptides of the present invention may be prepared in accordance with well-known procedures for preparing peptides from their constituent amino acids, which will be described in more detail below. The unusual amino acid, statine,

may be prepared in accordance with the procedure described by Rich et. al., J. Org. Chem. 43: 3624 (1978).

A general method of preparation may be described in the following terms:

A method of preparing a peptide of formula I, said peptide being comprised of from three to five amino acids identified as I through V, amino acid (AA) I being at the C-terminus of said peptide, to which substituents B-D-E are attached, and amino acid (AA) V being at the N-terminus of said peptide, to which substituent A is attached, comprising the steps of:

(A) treating the desired ester or amide of the C-terminus amino acid (AA I) with the next adjacent amino acid (AA II) of said peptide, the amino group of said amino acid being protected by a protecting group, in the presence of a condensing agent, whereby a dipeptide of the two amino acids (AA I and II) is formed;

(B) deprotecting the dipeptide formed in Step (A) by removing the protecting group from the amino group of AA II;

(C) treating the dipeptide of AA I and AA II with AA III, the amino group of which is protected by a protecting group, in the presence of a condensing agent, whereby a tripeptide of AA I, AA II and AA III is formed;

(D) deprotecting the tripeptide formed in Step (C) by removing the protecting group from the amino group of AA III, to give the peptide of Formula I wherein A is hydrogen;

(E) treating the tripeptide formed in Step (D) where an ester of AAI is employed with hydrazine to give

the corresponding hydrazide, followed by treatment of said hydrazide with acidic nitrite to give the corresponding acyl azide, followed by treatment of said acyl azide with the appropriate amine compound to give the desired B substituent which is not an amino acid, in the peptide of Formula I; and optionally

(F) treating the tripeptide formed in Step (E) with $R_a^2\text{-}\underset{\underset{R_b^2}{|}}{X}\text{-}\overset{\overset{O}{\|}}{C}\text{-}W$, where X, R_a^2, and R_b^2, are as defined above and W is an acid halide, anhydride, or other carbonyl activating group, to give the peptide of Formula I wherein A is other than hydrogen; and optionally

(G) forming a quadripeptide up to a pentapeptide of AA I, through AA IV or AA V, by repeating the procedure of Step (C) using protected AA IV through protected AA V;

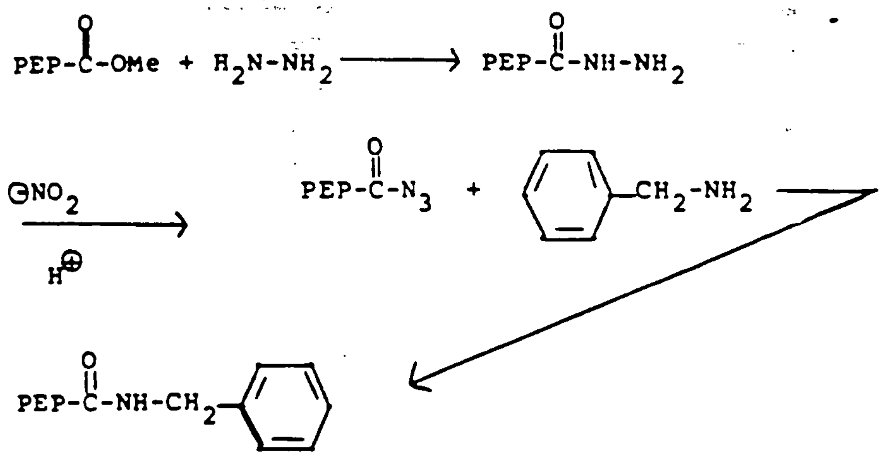
(H) deprotecting the quadripeptide through pentapeptide formed in Step (G) to give the peptide of Formula I wherein A is hydrogen; and optionally

(I) treating the quadripeptide through pentapeptide formed in Step (H) with $R_a^2\text{-}\underset{\underset{R_b^2}{|}}{X}\text{-}\overset{\overset{O}{\|}}{C}\text{-}W$, where X, R_a^2, and R_b^2 are as defined above and W is an acid halide, anhydride, or other carboxyl activating group, to give the peptide of Formula I wherein A is other than hydrogen;

said method also comprising, where necessary, prot c-tion of sidechain substituents of the component amino

acids AA I through AA V, with deprotection being carried out as a final step; said method also comprising any combination of the steps set out above, whereby the amino acids I through V and substituents A, B, D, and E are assembled in any desired order to prepare the peptide of Formula I; and said method also comprising employment of the steps set out above in a solid phase sequential synthesis, whereby in the initial step the carboxyl group of the selected amino acid is bound to a synthetic resin substrate while the amino group of said amino acid is protected, followed by removal of the protecting group, the succeeding steps being as set out above, the peptide as it is assembled being attached to said synthetic resin substrate; followed by a step of removing the peptide of Formula I from said synthetic resin substrate: (a) by strong acid cleavage to give E=OH; (b) by transesterification with a C_{1-4} alkanol to give E=O-C_{1-4}alkyl (followed by hydrolysis to give E=OH); or (c) by ammonolysis with NH_2R where R is hydrogen or C_{1-4}alkyl; said removal steps also optionally being carried out as treatments where solid phase sequential synthesis is not carried out, to give the same E substituent endings; and after removal of the peptide of Formula I from said synthetic resin substrate by transesterification to form the ester thereof as recited above, optionally the step of teating said ester thereof in accordance with the procedures described in Step (E) above to give the desired B substituent in the peptide of Formula I; removal of sidechain protecting groups being accomplished either before or after removal of the peptide of Formula I from said synthetic resin substrate.

Preparation of the peptides of Formula I having the desired B substituent, as described above in Step (E), may be illustrated as follows for the particular case where B=benzylamide (and PEP represents the remaining portion of the peptide of Formula I):



The phenyl analog of statine, (3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA) can be prepared in accordance with the procedure described by Rich et al., J. Med. Chem. 23: 27-33 (1980).

Other analogs of statine may be prepared in a straightforward manner. For example, the cyclohexylalanine analog of statine, (3S, 4S)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA) can be prepared by catalytic hydrogenation (using H_2/Rh on alumina, or other suitable catalyst) of the BOC-AHPPA, prepared as described in the paragraph immediately above. Alternatively, this and similar statine analogs can be prepared in accordance with the procedure described for statine, where the BOC-Leu starting material is replaced with the amino acid containing the desired

side chain. Thus, BOC-ACHPA can also be prepared starting from BOC-L-cyclohexylalanine, itself prepared, for example, by catalytic reduction of BOC-Phe, in the same manner as described for BOC-AHPPA.

The novel inhibitory peptides of the present invention are prepared by using the solid phase sequential synthesis technique.

In the following description several abbreviated designations are used for the amino acid components, certain preferred protecting groups, reagents and solvents. The meanings of such abbreviated designations are given below in Table I.

TABLE I

Abbreviated Designation	Amino Acid
AHPPA	(3S,4S)-4-amino-3-hydroxy-5-phenylpentanoic acid
ACHPA	(3S,4S)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid
Ala	L-alanine
Arg	L-arginine
Gly	L-glycine
His	D or L-histidine
Ile	L-isoleucine
Leu	L-leucine
Lys	L-lysine
Met	L-methionine
Orn	L-ornithine
Phe	L-phenylalanine
Ser	L-serine
Sar	L-sarcosine (N-methylglycine)
Sta	(3S,4S)-statine
Thr	L-threonine
Trp	L-tryptophan
Tyr	L-tyrosine
Val	L-valine

Abbreviated Designation	Protecting Groups
BOC	tert-butyloxycarbonyl
CBZ	benzyloxycarbonyl
DNP	dinitrophenyl
OMe	methyl ester

Abbreviated Designation	Activating Groups
HBT	1-hydroxybenzotriazole

Abbreviated Designation	Condensing Agents
DCCI	dicyclohexylcarbodiimide
DPPA	diphenylphosphorylazide

Abbreviated Designation	Reagents
TEA	triethylamine
TFA	trifluoroacetic acid

Abbreviated Designation	Solvents
A	ammonium hydroxide (conc.)
AcOH	acetic acid
C	chloroform
DMF	dimethylformamide
E	ethyl acetate
M	methanol
P	pyridine
THF	tetrahydrofuran
W	water

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The synthesis of the peptides of the present invention by the solid phase technique is conducted in a stepwise manner on chloromethylated resin. The resin is composed of fine beads (20-70 microns in diameter) of a synthetic resin prepared by copolymerization of styrene with 1-2 percent divinylbenzene. The benzene rings in the resin are chloromethylated in a Friedel-Crafts reaction with chloromethyl methyl ether and stannic chloride. The Friedel-Crafts reaction is continued until the resin contains 0.5 to 5 mmoles of chlorine per gram of resin.

The amino acid selected to be the C-terminal amino acid of the linear peptide is converted to its amino protected derivative. The carboxyl group of the selected C-terminal amino acid is bound covalently to the insoluble polymeric resin support, as for example, as the carboxylic ester of the resin-bonded benzyl chloride present in chloromethyl-substituted polystyrene-divinylbenzene resin. After the amino protecting group is removed, the amino protected derivative of the next amino acid in the sequence is added along with a coupling agent, such as dicyclohexylcarbodiimide. The amino acid reactant may be employed in the form of a carboxyl-activated amino acid such as ONP ester, an amino acid azide, and the like. Deprotection and addition of successive amino acids is performed until the desired linear peptide is formed.

The selection of protecting groups is, in part, dictated by particular coupling conditions, in part by the amino acid and peptide components involved in the reaction.

Amino-protecting groups ordinarily employed include those which are well known in the art, for example, urethane protecting substituents such as benzyloxy-carbonyl (carbobenzoxy), p-methoxycarbobenzoxy, p-nitrocarbobenzoxy, t-butyoxycarbonyl, and the like. It is preferred to utilize t-butyloxycarbonyl (BOC) for protecting the α-amino group in the amino acids undergoing reaction at the carboxyl end of said amino acid. The BOC protecting group is readily removed following such coupling reaction and prior to the subsequent step by the relatively mild action of acids (i.e. trifluoroacetic acid, or hydrogen chloride in ethyl acetate).

The OH group of Thr and Ser can be protected by the Bzl group and the -amino group of Lys can be protected by the INOC group or the 2-chlorobenzyloxycarbonyl (2-Cl-CBZ) group. Neither group is affected by TFA, used for removing BOC protecting groups. After the peptide is formed, the protective groups, such as 2-Cl-CBZ and Bzl, can be removed by treatment with HF or by catalytic hydrogenation.

After the peptide has been formed on the solid phase resin, it may be removed from the resin by a variety of methods which are well known in the art. For example the peptide may be cleaved from the resin with hydrazine, by ammonia in methanol, or by methanol plus a suitable base.

Preparation of the novel inhibitory peptides of the present invention utilizing the solid phase technique is illustrated in the following examples, which however, are not intended to be any limitation of the present invention.

EXAMPLE 1

Phenoxyacetyl-L-histidyl-(3S,4S)-AHPPA-L-leucyl-benzylamide

The title peptide was prepared by standard solid phase methodology, as described in Erickson and Merrifield, Proteins, 3rd et., 2:257-527, 1976, using a Beckman Model 990B peptide synthesizer to carry out the operations according to the attached programs.

A. Phenoxyacetyl-L-histidyl-(3S,4S)-AHPPA-L-leucyl-O-Resin

The starting polymer was BOC-Leu esterified to 2% cross-linked polystyrene-divinylbenzene (≈2 mmol, 2.00 g). The N^{α}-BOC derivatives of Sta, and His-DNP, ~~Phe, and Pro~~ were coupled using dicyclohexylcarbodiimide with an equivalent of the additive 1-hydroxybenzotriazole hydrate. The Sta was prepared in accordance with Rich et al., J. Org. Chem. 43:3624, 1978. The BOC-group was removed with 40% trifluoroacetic acid. A coupling of 60 minutes followed by a recoupling of 120 minutes (2.5 equivalents each time of BOC-amino acid) were used for each amino acid, except for Sta. These coupling times had been previously demonstrated to give complete coupling (as judged by the method of Kaiser) in this sequence. In order to conserve the amounts of Sta employed, an initial coupling using 1.08 equivalents of N^{α}-BOC-AHPPA (in 20 ml of 1:1 CH_2Cl_2/DMF) for 72 hrs gave approximately 95% complete reaction. The addition of an additional 0.12 equivalents of N^{α}-BOC-AHPPA plus an equal amount of DCCI to the stirring suspension gave compl te c upling after an additional 18 hrs. The N-terminal

phenoxyacetyl group was coupled for 60 minutes as the symmetrical anhydride generated in situ from 5.0 equivalents of phenoxyacetic acid and 2.5 equivalents of DCCI. This was followed by a recoupling for 120 minutes using 2.5 equivalents of phenyoxyacetic acid, HBT, and DCCI. The DNP protecting group on His was removed in the final program using two 25-minute treatments with 10% thiophenol in DMF. The finished resin-peptide (2.9 g) was dried and suspended in 40 ml of dry methanol.

SCHEDULE OF STEPS FOR 2 MMOL RUN

Step	Solvent/Reagent	Vol. (ml)	Mix time (min)
Coupling Program 1			
1	CH_2Cl_2	6 x 20	2
2	40% TFA in CH_2Cl_2	1 x 20	2
3	40% TFA in CH_2Cl_2	1 x 20	25
4	CH_2Cl_2	3 x 20	2
5	10% TEA in CH_2Cl_2	2 x 20	5
6	CH_2Cl_2	3 x 20	2
7	BOC-amino acid, HBT in 1:1 DMF/CH_2Cl_2	20	5
8	1.0M DCCI in CH_2Cl_2	5	60
9	DMF	1 x 20	2
10	MeOH	2 x 20	2
11	CH_2Cl_2	1 x 20	2

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Re-Couple Program 2

1	CH_2Cl_2	1 x 20	2
2	10% TEA in CH_2Cl_2	2 x 20	5
3	CH_2Cl_2	3 x 20	2
4	BOC-amino acid, HBT in 1:1 DMF/CH_2Cl_2	40	5
5	1.0M DCCI in CH_2Cl_2	5	120
6	DMF	1 x 20	2
7	MeOH	2 x 20	2
8	CH_2Cl_2	5 x 20	2

Program 3 (DNP removal)

1	CH_2Cl_2	1 x 20	2
2	DMF	2 x 20	2
3	10% phenylthiol in DMF	1 x 20	25
4	DMF	1 x 20	2
5	10% TEA in CH_2Cl_2	1 x 20	2
6	DMF	2 x 20	2
7	10% phenylthiol in DMF	1 x 20	25
8	DMF	3 x 20	2
9	MeOH	2 x 20	2
10	CH_2Cl_2	2 x 20	2
11	MeOH	2 x 20	2
12	CH_2Cl_2	2 x 20	2
13	MeOH	2 x 20	2

B. Phenoxyacetyl-L-histidyl-(3S,4S)-AHPPA-L-leucine methyl ester

To the suspension prepared in Step A above was added 10 ml diisopropylethylamine, and the reacti n mixture was stirred under a dry nitrogen

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atmosphere for 18 hours. The reaction mixture was then filtered and the yellow solution was evaporated under reduced pressure to give 1.4 g of crude methyl ester. This crude product was dissolved in 50 ml of methylene chloride and washed with water. The methylene chloride layer was dried over sodium sulfate and evaporated to give 1.0 g of yellow powder. This material was chromatographed on a silica column (160 g, 0.04-0.063 mmol) packed and eluted with chloroform/methanol/water/acetic acid - 120:10:1.6:0.4. The pure methyl ester was obtained by evaporation of the appropriate fractions and precipitation from 3 ml of methylene chloride/50 ml of petroleum ether. Yield was 0.77 g.

C. Phenoxyacetyl-L-histidyl-(3S, 4S)-AHPPA-L-leucine hydrazide

A portion (0.44 g) of the ester prepared in Step B above was converted to the hydrazide by dissolving it in 2 ml of a 1:1 mixture of dry methanol and anhydrous hydrazine. After a few minutes the solution was evaporated to dryness, at 30°C, and the crude hydrazide was dissolved in 15 ml of n-butanol and washed 5 times with an equal volume of water containing a small amount of sodium chloride. The n-butanol layer was then evaporated and the hydrazide was precipitated from methylene chloride/petroleum ether. The yield was 0.40 g of a single material by thin layer chromatography (silica).

The material was over 99% pure as determined by high performance liquid chromatography.

D. Phenoxyacetyl-L-histidyl-(3S,4S)-AHPPA-L-leucyl-benzylamide

The hydrazide prepared in Step C above can be converted by acidic nitrite to its corresponding acyl azide, and coupled to a wide variety of amines. Thus, ~~0.10~~ 0.06 g of the hydrazide (0.10 mmol) was dissolved in 0.5 ml of dry, degassed dimethylformamide, and cooled to -30°C under a nitrogen atmosphere. To this cold solution was added 1.1 mmol of fresh 7.1 N hydrochloric acid in dry tetrahydrofuran. To this acidic solution was then added 15μl of isoamylnitrite over a period of 10 minutes. After approximately 1 hour, there was added 1.1 mmol of diisopropylethylamine, and the pH was adjusted with additional base to 7 (using pH 6-8 paper). To this solution of the acyl azide was added 2 equivalents (0.2 mmol) of benzylamine, and the reaction mixture was held at -20°C for 18 hours. The reaction solution was evaporated, and the resulting yellow oil was triturated with ether. The remaining solid was dissolved in 10 ml of *n*-butanol and washed twice with water, once with 5% sodium bicarbonate, and twice with sodium chloride solution. The butanol layer was then evaporated, dissolved in 10 ml methylene chloride, and the product was precipitated with 50 ml of ether. The yield was 60 mg showing a single spot on thin layer chromatography. The

product was 90.7% pure, as determined by high performance liquid chromatography, and had an acceptable amino acid analysis: His 1.00, Phe 1.01, and Leu 1.01. A 300 MHz 'HNMR spectrum was consistent with the desired structure.

EXAMPLE 2-27

Following the standard solid phase methodology described above in Example 1, additional inhibitory peptides of the present invention were prepared. The peptides prepared are set out in the following table. Satisfactory amino acid analyses were obtained by Spinco method for each listed peptide.

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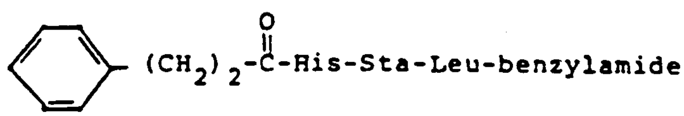
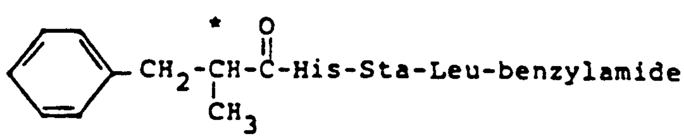
Exm.
No. Peptide

2. His-Sta-Leu-Phe-methyl ester;
3. His-Sta-Phe-Phe-amide;
4. His-Sta-Ala-Phe-amide;
5. BOC -His-Sta-Leu-Phe-amide;
6. POA -His-Sta-Leu-Phe-methyl ester;
7. POA -His-Sta-Leu-Phe-amide;
8. POA -His-Sta-Phe-Phe-amide;

9. 

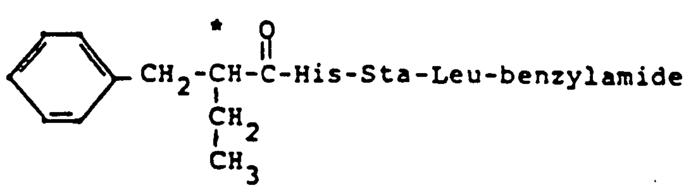
[3-phenylpropionyl-]

10. 

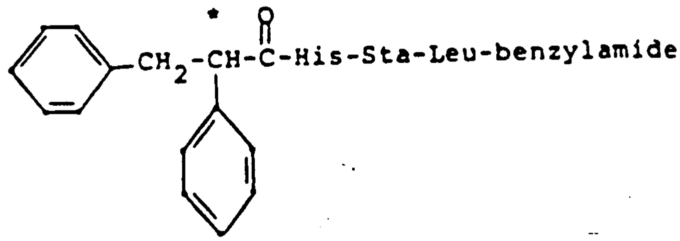
[2-methyl-3-phenylpropionyl-] (Isomer"A")

11. 

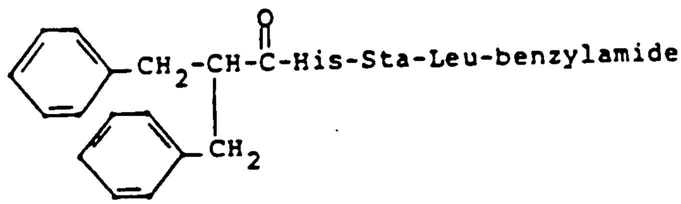
[2-methyl-3-phenylpropionyl-] (Isomer"B")

12. 

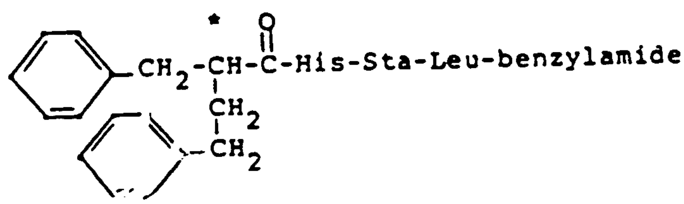
[2-ethyl-3-phenylpropionyl-] (1:1 Mixture of isomers)

13. 

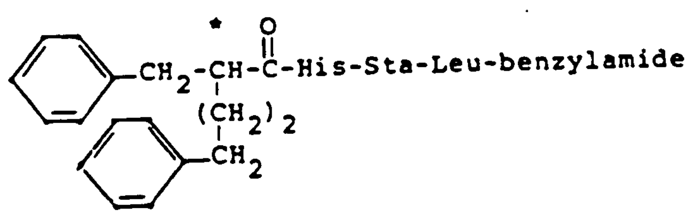
[2,3-diphenylpropionyl-] (1:1 Mixture of isomers)

14. 

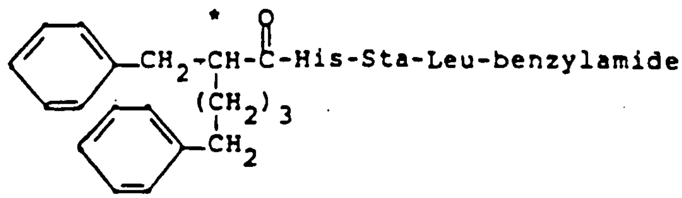
[2-benzyl-3-phenylpropionyl-]

15. 

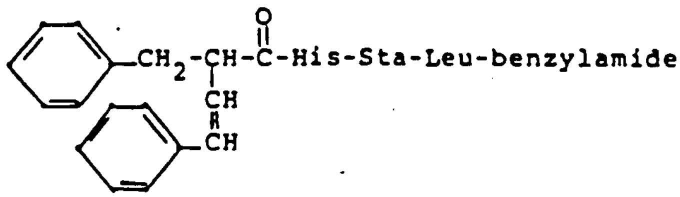
[2-[illegible]enethyl-3-phenylpropionyl-] (1:1 Mixture of isomers)

16. 

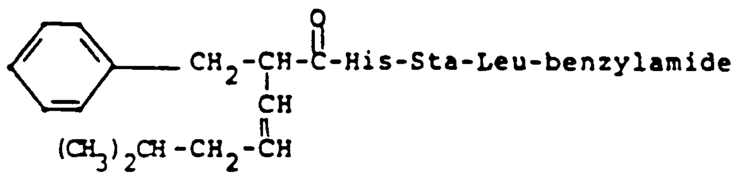
[2-phenylpropyl-3-phenylpropionyl-] (1:1 Mixture of isomers

17. 

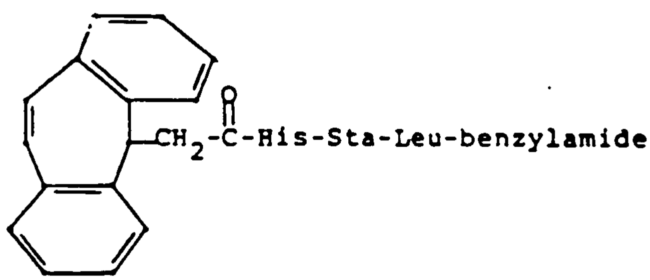
[2-phenylbutyl-3-phenylpropionyl-] (1:1 Mixture of isomers)

18. 

[2-phenethenyl-3-phenylpropionyl-]

19. 

[2-(4,4-dimethyl-1-butenyl)-3-phenylpropionyl-]

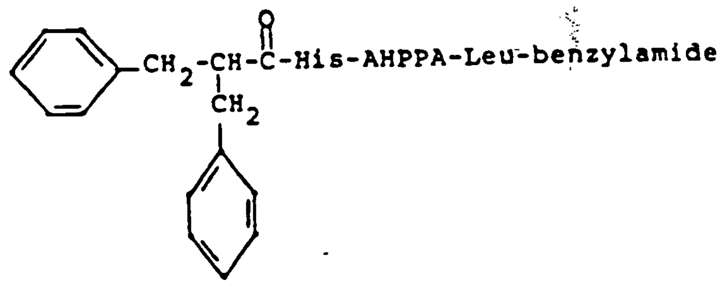
20. 

[10,11-dihydro-5H-dibenzo[a,d]cycloheptenylcarbonyl-]

21. BOC-His-AHPPA-Leu-benzylamide

22. 

[3-phenylpropionyl-]

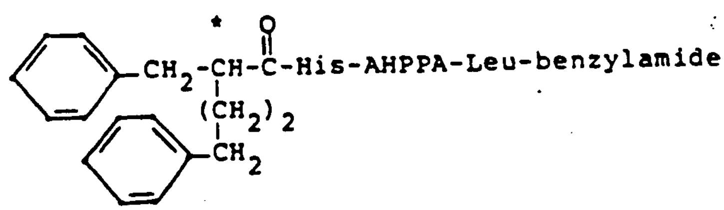
23. 

[2-benzyl-3-phenylpropionyl-]

24. POA-His-ACHPA-Leu-Phe-amide

25. POA-His-ACHPA-Ile-benzylamide

26. POA-His-ACHPA-Ile-2-pyridylmethylamide

27. 

[2-phenylpropyl-3-phenylpropionyl-] (1:1 Mixture of isome

For the peptides prepared above, various analytical methods were carried out to verify the structure of the peptide products. The following table indicates which methods were employed and summarizes the results where practicable.

Example No.	Analytical Method			
	TLC[1] (No. of systems)	HPLC[2]	AA[3]	NMR[4]
2	95.0%	95.0%	X	X
3	96.0%	96.0%	X	X
4	99.0%	92.0%	X	X
5	95.0%	95.0%	X	X
6	95.0%	98.2%	X	X
7	98.0%	98.3%	X	X
8	95.0%	92.1%	X	X
9	95.0%	98.1%	X	X
10	95.0%	94.8%	X	X
11	95.0%	90.1%	X	X
12	95.0%	97.2%	X	X
13	95.0%	99.0%	X	X
14	95.0%	97.5%	X	X
15	95.0%	92.0%	X	X
16	95.0%	97.0%	X	X
17	95.0%	93.2%	X	X
18	95.0%	93.3%	X	X
19	95.0%	97.0%	X	X
20	95.0%	97.0%	X	X

21	95.0%	97.8	X	X
22	95.0%	94.6%	X	X
23	95.0%	98.2%	X	X
24	98.0%	98.6%	X	X
25	90.0%	87.0%	X	X
26	95.0%	95.0%	X	X
27	95.0%	87.8%	X	X

[1]TLC = thin layer chromatography on silica gel; visualization by reagents which tend to detect peptides; % refers to estimated purity.

[2]HPLC = high pressure liquid chromatography; detection by ultraviolet absorption at 240 nm or 210 nm; chromatography is reverse phase, values should be 1.00 $\pm$ 0.03.

[3]AA = amino acid analysis; peptides are hydrolyzed to their component amino acids, which are then quantitatively measured; values should be 1.00 $\pm$ 0.03.

[4]NMR = nuclear magnetic resonance spectroscopy at 300 MHz or 360 MHz for protons; X = spectrum consistent with structure; ___ = not performed.

EXAMPLE 28

Hog Renin Inhibition

An assay was carried out in order to determine the inhibitory potency of the peptides of the present invention. The assay measured the inhibition of hog kidney renin, and was in accordance with the procedure described in Rich et al., J. Med. Chem. 23:27, 1980, except that a pH of 7.3 was used.

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The results of the assay, illustrated in the table below, are expressed as I_{50} values, which refers to the concentration of peptide inhibitor necessary to produce 50% inhibition of renin activity. This I_{50} value is obtained typically by plotting data from four inhibitor concentrations. Pepstatin was used as an active control.

Peptide	I_{50}(M)
His-Sta-Leu-Phe-methyl ester	1.4×10^{-5}
His-Sta-Phe-Phe-methyl ester	4×10^{-4}
His-Sta-Ala-Phe-methyl ester	5% at 10^{-4}
BOC -His-Sta-Leu-Phe-amide	4×10^{-4}
POA -His-Sta-Leu-Phe-methyl ester	3.3×10^{-6}
POA -His-Sta-Leu-Phe-amide	7.6×10^{-6}
POA -His-Sta-Phe-Phe-amide	4.0×10^{-5}
3-phenylpropionyl-His-Sta-Leu-benzylamide	2% at 10^{-7}
	15% at 1.3×10^{-6}
	("A" Isomer)
2-methyl-3-phenylpropionyl-His-Sta-Leu-benzylamide	9.4×10^{-7}
	("B" Isomer)
2-ethyl-3-phenylpropionyl-His-Sta-Leu-benzylamide	1.4×10^{-6}
2,3-diphenylpropionyl-His-Sta-Leu-benzylamide	2.2×10^{-6}
2-benzyl-3-phenylpropionyl-His-Sta-Leu-benzylamide	1.5×10^{-7}
2-phenethyl-3-phenylpropionyl-His-Sta-Leu-benzylamide	3.7×10^{-7}
2-phenylpropyl-3-phenylpropionyl-His-Sta-Leu-benzylamide	3.1×10^{-8}
2-phenylbutyl-3-phenylpropionyl-His-Sta-Leu-benzylamide	5.2×10^{-7}
2-phenethenyl-3-phenylpropionyl-His-Sta-Leu-benzylamide	2.6×10^{-7}

2-(4,4-dimethyl-1-butenyl)-3-phenylpropionyl-His-Sta-Leu-benzylamide	6.1×10^{-7}
10,11-dihydro-5H-dibenzo-[2,d]cycloheptenylcarbonyl-His-Sta-Leu-benzylamide	1.1×10^{-5}
BOC -His-AHPPA-Leu-benzylamide	- at 10^{-6}
3-phenylpropionyl-His-AHPPA-Leu-benzylamide	3.5×10^{-8}
POA-His-AHPPA-Leu-benzylamide	2.0×10^{-8}
2-benzyl-3-phenylpropionyl-His-AHPPA-Leu-benzylamide	2.6×10^{-9}
POA-His-ACHPA-Leu-Phe-amide	5.1×10^{-7}
POA-His-ACHPA-Ile-benzylamide	2.9×10^{-7}
POA-His-ACHPA-Ile-2-pyridylmethylamide	1.4×10^{-6}
2-phenylpropyl-3-phenylpropionyl-His-AHPPA-Leu-benzylamide	8.1×10^{-9}

EXAMPLE 29

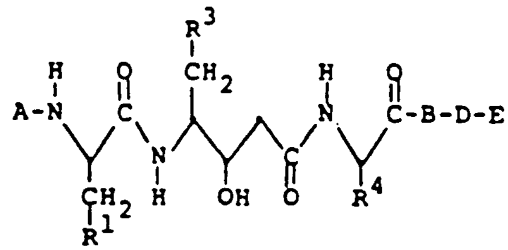
Human Renin Inhibition

An assay was carried out in order to determine the inhibitory potency of the peptides of the present invention. The assay measured the inhibition of human kidney renin purified as described in Bangham, D. R., Robertson, I., Robinson, J. I. S., Robinson, C. J., and Tree, M., Clinical Science and Molecular Medicine, 48 (Supp. 2): 136s-159s (1975), and further purified by affinity chromatography on pepstatin-aminohexyl-Sepharase as described in Poe, M., Wu., J. K., Florance, J. R., Radkey, J. A., Bennett, C. D., and Hoagsteen, K., J. Biol. Chem. (1982, in press). The assay was also in accordance with Poe *et al*. cited above. Results are expressed as K_I values, which refer to the dissociation constant of the inhibited enzyme - inhibitor complex. This K_I value was obtained as described above. Pepstatin was used as an active control. The results are set out in the table below.

Peptide	K_I(M)
2,3-diphenylpropionyl-His-Sta-Leu-benzylamide	9×10^{-7}
2-benzyl-3-phenylpropionyl-His-Sta-Leu-benzylamide	8.3 x 10-[illegible]
2-phenylpropyl-3-phenylpropionyl-His-Sta-Leu-benzylamide	9.7 x 10-[illegible]
2-phenylbutyl-3-phenylpropionyl-His-Sta-Leu-benzylamide	1.7 x 10-[illegible]
3-phenylpropionyl-His-AHPPA-Leu-benzylamide	7.1 x 10-[illegible]
POA-His-AHPPA-Leu-benzylamide	1.0 x 10-[illegible]
2-benzyl-3-phenylpropionyl-His-AHPPA-Leu-benzylamide	3.4 x 10-[illegible]
POA-His-ACHPA-Leu-Ph -amide	1.1 x 10-[illegible]
2-phenylpropyl-3-phenylpropionyl-His-AHPPA-Leu-benzylamide	2.3 x 10-[illegible]

WHAT IS CLAIMED IS:

1. A peptide of the formula:



(I.)

wherein:

A is hydrogen; or $R^2_a\text{-}X(R^2_b)\text{-}C(=O)\text{-}$

where

X is -O-; -O-CH-; -CH-O-; -CH-; -NH-CH-; or -S-CH-; and

R^2_a and R^2_b may be the same or different and are hydrogen; $Y\text{-}(CH_2)_n\text{-}$ or $Y\text{-}(CH_2)_m\text{-}CH{=}CH\text{-}(CH_2)_p\text{-}$, where Y is hydrogen; aryl; aryl substituted with up to five members independently selected from the group consisting of C_{1-8}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, and halo;

n is 0 to 5; m is 0 to 2; and p is 0 to 2; except that where X is -O-, only one of R^2_a or R^2_b is present;

R^1 is hydrogen; C_{1-4} alkyl, provided, that where R^1 is <u>i</u>-propyl, and B is Phe or Tyr, A is other than hydrogen or phenoxyacetyl; hydroxy C_{1-4} alkyl; aryl; aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy; fluoro, chloro, bromo, and iodo; indolyl; 4-imidazolyl; amine C_{2-4} alkyl; guanidyl C_{2-3} alkyl; or methylthiomethyl;

R^3 is C_{3-6} alkyl; C_{3-7} cycloalkyl; aryl; or C_{3-7}cycloalkyl or aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, fluoro, chloro, bromo, and iodo;

R^4 is hydrogen; or $-\underset{\displaystyle R^2}{\underset{|}{C}H}-R^5$, where R^2 is hydrogen; C_{1-4}alkyl; aryl; aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, fluoro, chloro, bromo, and iodo; or indolyl;

R^5 is hydrogen; C_{1-4} alkyl; hydroxy; or C_{3-7} cycloalkyl; and

B is (1) $-Y-(CH_2)_n-R^6$

where

Y is -NH- or -O-;

n is 0 to 5; and

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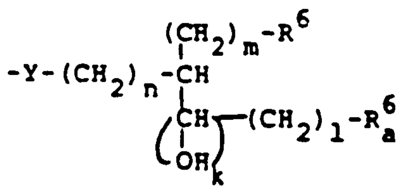
R^6 is hydrogen; hydroxy; C_{1-4}alkyl; C_{3-7}cycloalkyl; aryl; aryl substituted with up to five members independently selected from the group consisting of C_{1-6}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, amino, mono- or di- C_{1-4} alkylamino, and halo; amino; mono-, di-, or tri-C_{1-4}alkylamino; guanidyl; heterocyclic; or heterocyclic substituted with up to five members independently selected from the group consisting of C_{1-6}alkyl, hydroxy, trifluoromethyl, C_{1-4}alkoxy, halo, aryl, aryl C_{1-4}alkyl, amino, and mono- or di-C_{1-4}alkylamino;

(2) 

where

Y is as defined above;

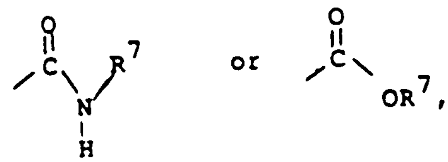
n is 0 or 1;

k is 0 or 1;

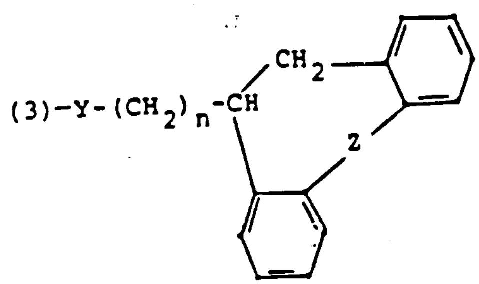
l is 1 to 4;

m is 1 to 4; and

R^6 and R^6_a may be the same or different and have the same meaning as R^6 above and R^6_a may additionally be



where R^7 is hydrogen or C_{1-3}alkyl;



where

Y is as defined above;

n is 0 or 1; and

Z is

(a) $-(CH_2)_n-\underset{R^7}{\underset{|}{CH}}-$

where

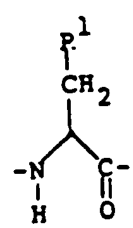
n is 0 or 1; and

R^7 is as defined above; or

(b) $-(CH_2)_n-\underset{CH_2}{\underset{\|}{C}}-$

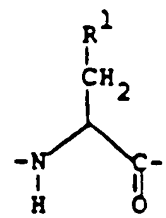
where

n is 0 or 1; or



;

D is absent; glycyl; sarcosyl; or



; and

E is absent; OR; NHR; or $N(R)_2$, where R may be the same or different and is hydrogen or C_{1-4}alkyl;

wherein all of the asymmetric carbon atoms have an S configuration, except for those in the A, B, and D substituents, which may have an S or R configuration; and a pharmaceutically acceptable salt thereof.

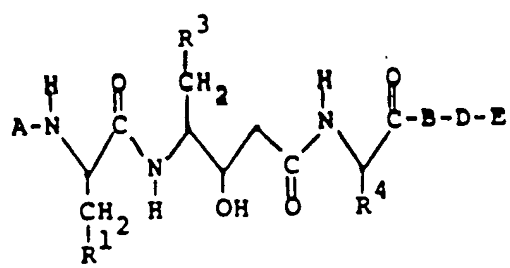
2. A peptide according to Claim 1 wherein the peptide is a member selected from the group consisting essentially of:

His-Sta-Leu-Phe-methyl ester;
His-Sta-Phe-Phe-methyl ester;
His-Sta-Ala-Phe-methyl ester;
BOC-His-Sta-Leu-Phe-amide;
POA-His-Sta-Leu-Phe-methyl ester;
POA-His-Sta-Leu-Phe-amide;
POA-His-Sta-Phe-Phe-amide;
3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-methyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-ethyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2,3-diphenylpropionyl-His-Sta-Leu-benzylamide;
2-benzyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenethyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenylpropyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenylbutyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;

2-phenethenyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-(4,4-dimethyl-1-butenyl)-3-phenylpropionyl-His-Sta-Leu-benzylamide;
10,11-dihydro-5H-dibenzo-[2,d]cycloheptenylcarbonyl-His-Sta-Leu-benzylamide;
BOC -His-AHPPA-Leu-benzylamide;
3-phenylpropionyl-His-AHPPA-Leu-benzylamide;
POA-His-AHPPA-Leu-benzylamide;
2-benzyl-3-phenylpropionyl-His-AHPPA-Leu-benzylamide.

POA-His-ACHPA-Leu-Phe-amide;
POA-His-ACHPA-Ile-benzylamide;
POA-His-ACHPA-Ile-2-pyridylmethylamide;
2-phenylpropyl-3-phenylpropionyl-His-AHPPA-Leu-benzylamide.

3. A pharmaceutical composition for treating renin-associated hypertension, comprising a pharmaceutical carrier and a therapeutically effective amount of a peptide of the formula:



(I.)

wherein:

A is hydrogen; or $R_a^2\text{-}\underset{\underset{R_b^2}{|}}{X}\text{-}\overset{\overset{O}{\|}}{C}\text{-}$

where

X is -O-; -O-CH-; -CH-O-; -CH-; -NH-CH-; or -S-CH-; and

R_a^2 and R_b^2 may be the same or different and are hydrogen; $Y\text{-}(CH_2)_n\text{-}$ or $Y\text{-}(CH_2)_m\text{-}CH{=}CH\text{-}(CH_2)_p\text{-}$, where Y is hydrogen; aryl; aryl substituted with up to five members independently selected from the group consisting of C_{1-8}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, and halo;

n is 0 to 5; m is 0 to 2; and p is 0 to 2; except that where X is -O-, only one of R_a^2 or R_b^2 is present;

R^1 is hydrogen; C_{1-4} alkyl, provided, that where R^1 is i-propyl, and B is Phe or Tyr, A is other than hydrogen or phenoxyacetyl; hydroxy C_{1-4} alkyl; aryl; aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy; fluoro, chloro, bromo, and iodo; indolyl; 4-imidazolyl; amine C_{2-4} alkyl; guanidyl C_{2-3} alkyl; or methylthiomethyl;

R^3 is C_{3-6} alkyl; C_{3-7} cycloalkyl; aryl; or C_{3-7}cycloalkyl or aryl substituted with up to three members selected from the group

consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, fluoro, chloro, bromo, and iodo;

R^4 is hydrogen; or $-\underset{\substack{| \\ R^2}}{CH}-R^5$, where R^2 is hydrogen; C_{1-4}alkyl; aryl; aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, fluoro, chloro, bromo, and iodo; or indolyl;

R^5 is hydrogen; C_{1-4} alkyl; hydroxy; or C_{3-7} cycloalkyl; and

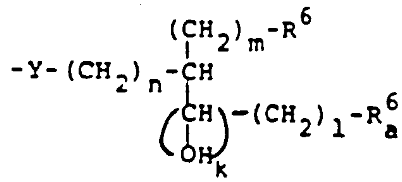
B is (1) $-Y-(CH_2)_n-R^6$

where

Y is -NH- or -O-;

n is 0 to 5; and

R^6 is hydrogen; hydroxy; C_{1-4}alkyl; C_{3-7}cycloalkyl; aryl; aryl substituted with up to five members independently selected from the group consisting of C_{1-6}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, amino, mono- or di- C_{1-4} alkylamino, and halo; amino; mono-, di-, or tri-C_{1-4}alkylamino; guanidyl; heterocyclic; or heterocyclic substituted with up to five members independently selected from the group consisting of C_{1-6}alkyl, hydroxy, trifluoromethyl, C_{1-4}alkoxy, halo, aryl, aryl C_{1-4}alkyl, amino, and mono- or di-C_{1-4}alkylamino;

(2) 

where

Y is as defined above;

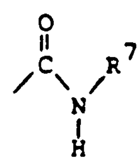
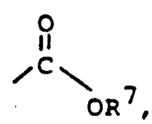
n is 0 or 1;

k is 0 or 1;

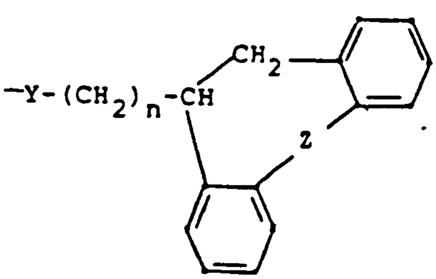
l is 1 to 4;

m is 1 to 4; and

R^6 and R^6_a may be the same or different and have the same meaning as R^6 above and R^6_a may additionally be

 or 

where R^7 is hydrogen or C_{1-3}alkyl;

(3) 

where

Y is as defined above;

n is 0 or 1; and

Z is

(a) $-(CH_2)_n-\underset{|\;R^7}{CH}-$

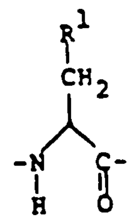
where

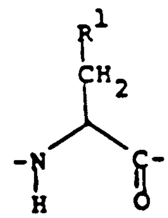
n is 0 or 1; and

R^7 is as defined above; or

(b) $-(CH_2)_n-\underset{\|\;CH_2}{C}-$

where

n is 0 or 1; or  ;

D is absent; glycyl; sarcosyl; or  ; and

E is absent; OR; NHR; or $N(R)_2$, where R may be the same or different and is hydrogen or C_{1-4}alkyl;

wherein all of the asymmetric carbon atoms have an S configuration, except for those in the A,-B,and D substituents, which may have an S or R configuration; and a pharmaceutically acceptable salt thereof.

4. A composition according to Claim 3 wherein the peptide is a member selected from the gr up consisting essentially f:

His-Sta-Leu-Phe-methyl ester;
His-Sta-Phe-Phe-methyl ester;
His-Sta-Ala-Phe-methyl ester,
BOC-His-Sta-Leu-Phe-amide;
POA-His-Sta-Leu-Phe-methyl ester;
POA-His-Sta-Leu-Phe-amide;
POA-His-Sta-Phe-Phe-amide;
3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-methyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-ethyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2,3-diphenylpropionyl-His-Sta-Leu-benzylamide;
2-benzyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenethyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenylpropyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenylbutyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-phenethenyl-3-phenylpropionyl-His-Sta-Leu-benzylamide;
2-(4,4-dimethyl-1-butenyl)-3-phenylpropionyl-His-Sta-Leu-benzylamide;
10,11-dihydro-5H-dibenzo-[2,d]cycloheptenylcarbonyl-His-Sta-Leu-benzylamide;
BOC -His-AHPPA-Leu-benzylamide;
3-phenylpropionyl-His-AHPPA-Leu-benzylamide;
POA-His-AHPPA-Leu-benzylamide;
2-benzyl-3-phenylpropionyl-His-AHPPA-Leu-benzylamide;

POA-His-ACHPA-Leu-Phe-amide;
POA-His-ACHPA-Ile-benzylamide;
POA-His-ACHPA-Ile-2-pyridyl-methylamide;
2-phenylpropyl-3-phenylpropionyl-His-AHPPA-Leu-benzylamide.

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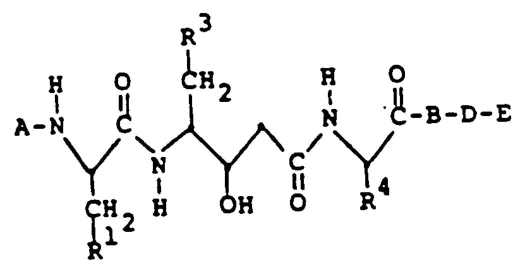
5. A pharmaceutical composition for treating renin-associated hyperaldosteronism, comprising a pharmaceutical carrier and a therapeutically effective amount of a peptide of the formula



(I.)

wherein:

A is hydrogen; or $R_a^2\text{-}\underset{\substack{| \\ R_b^2}}{X}\text{-}\overset{\substack{O \\ \|}}{C}\text{-}$

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where

X is -O-; -O-CH-; -CH-O-; -CH-; -NH-CH-; ;or -S-CH-; and

R_a^2 and R_b^2 may be the same or different and are hydrogen; $Y-(CH_2)_n-$ or $Y-(CH_2)_m-CH=CH-(CH_2)_p-$, where Y is hydrogen; aryl; aryl substituted with up to five members independently selected from the group consisting of C_{1-8}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, and halo;

n is 0 to 5; m is 0 to 2; and p is 0 to 2; except that where X is -O-, only one of R_a^2 or R_b^2 is present;

R^1 is hydrogen; C_{1-4} alkyl, provided, that where R^1 is i-propyl, and B is Phe or Tyr, A is other than hydrogen or phenoxyacetyl; hydroxy C_{1-4} alkyl; aryl; aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy; fluoro, chloro, bromo, and iodo; indolyl; 4-imidazolyl; amine C_{2-4} alkyl; guanidyl C_{2-3} alkyl; or methylthiomethyl;

R^3 is C_{3-6} alkyl; C_{3-7} cycloalkyl; aryl; or C_{3-7}cycloalkyl or aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, fluoro, chloro, bromo, and iodo;

R^4 is hydrogen; or $-\underset{\substack{| \\ R^2}}{C}H-R^5$, where R^2 is hydrogen; C_{1-4}alkyl; aryl; aryl substituted with up to three members selected from the group consisting of C_{1-4}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, fluoro, chloro, bromo, and iodo; or indolyl;

R^5 is hydrogen; C_{1-4} alkyl; hydroxy; or C_{3-7} cycloalkyl; and

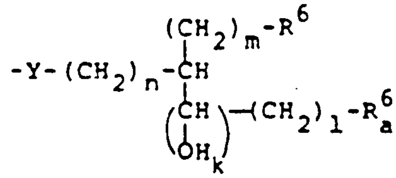
B is (1) $-Y-(CH_2)_n-R^6$

where

Y is -NH- or -O-;

n is 0 to 5; and

R^6 is hydrogen; hydroxy; C_{1-4}alkyl; C_{3-7}cycloalkyl; aryl; aryl substituted with up to five members independently selected from the group consisting of C_{1-6}alkyl, trifluoromethyl, hydroxy, C_{1-4}alkoxy, amino, mono- or di- C_{1-4} alkylamino, and halo; amino; mono-, di-, or tri-C_{1-4}alkylamino; guanidyl; heterocyclic; or heterocyclic substituted with up to five members independently selected from the group consisting of C_{1-6}alkyl, hydroxy, trifluoromethyl, C_{1-4}alkoxy, halo, aryl, aryl C_{1-4}alkyl, amino, and mono- or di-C_{1-4}alkylamino;

(2) 

where

Y is as defined above;

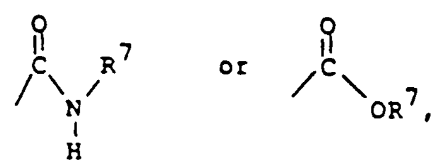
n is 0 or 1;

k is 0 or 1;

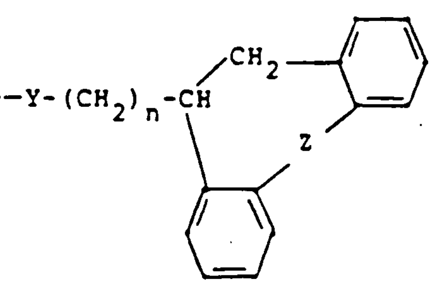
l is 1 to 4;

m is 1 to 4; and

R^6 and R^6_a may be the same or different and have the same meaning as R^6 above and R^6_a may additionally be



where R^7 is hydrogen or C_{1-3}alkyl;

(3) 

where

Y is as defined above;

n is 0 or 1; and

Z is

(a) $-(CH_2)_n-\underset{R^7}{\underset{|}{CH}}-$

where

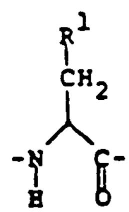
n is 0 or 1; and

R^7 is as defined above; or

(b) $-(CH_2)_n-\underset{CH_2}{\underset{\|}{C}}-$

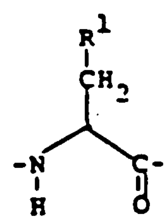
where

n is 0 or 1; or



;

D is absent; glycyl; sarcosyl; or



; and

E is absent; OR; NHR; or $N(R)_2$, where R may be the same or different and is hydrogen or C_{1-4}alkyl;

wherein all of the asymmetric carbon atoms have an S configuration, except for those in the A, B, and D substituents, which may have an S or R configuration; and a pharmaceutically acceptable salt thereof.